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Persistence and fate of 17β-estradiol and testosterone in agricultural soils

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Abstract

Steroidal hormones are constantly released into the environment by man-made and natural sources. The goal of this study was to examine the persistence and fate of 17β-estradiol and testosterone, the two primary natural sex hormones. Incubation experiments were conducted under aerobic and anaerobic conditions using [4-^{14}C]-radiolabeled 17β-estradiol and testosterone. The results indicated that 6% of 17β-estradiol and 63% of testosterone could be mineralized to $^{14}CO_2$ in native soils under aerobic conditions. In native soils under anaerobic conditions, 2% of testosterone and no 17β-estradiol was methanogenized to $^{14}CH_4$. Essentially, no mineralization of either testosterone or 17β-estradiol to $^{14}CO_2$ occurred in autoclaved soils under aerobic or anaerobic condition. Results also indicated that 17β-estradiol could be transformed to an unidentified polar compound through abiotic chemical processes; however, 17β-estradiol was only oxidized to estrone via biological processes. The TLC results also indicated that testosterone was degraded, not by physical–chemical processes but by biological processes. Results also indicated that the assumed risks of estrogenic hormones in the environment might be over-estimated due to the soil's humic substances, which can immobilize majority of estrogenic hormones, and thereby reduce their bioavailability and toxicity.

Keywords: 17β-estradiol; Testosterone; Hormones; Incubation experiment; Mineralization

1. Introduction

The primary, natural steroidal hormones in the environment include estradiol, estrone, testosterone (Fig. 1), and progesterone. The concern about these hormones is their ability to alter the sexual behavior and endocrine systems of animals and aquatic species (Larsson et al., 2000; Oshima et al., 2003; Teles et al., 2004). The two most studied hormones are 17β-estradiol and testosterone, which are classified as endocrine disrupting compounds. Most hormones are produced and released into the environment by humans (e.g., human urine and feces), livestock (e.g., animal manure), and wildlife (Shore et al., 1998; Shore and Shemesh, 2003). Johnson et al. (2000) reported that on an average, 1.6 μg d^{-1} of 17β-estradiol is excreted by human males and 2.3–2.5 μg d^{-1} of 17β-estradiol is excreted by females. Johnson et al. (2000) also reports that pregnant women can excrete up to 259 μg d^{-1} of 17β-estradiol. These human sources of hormones can enter the environment from wastewater and sewage treatment works (Desbrow et al., 1998; Jiang et al., 2005; Beck and Radke, 2006). Ternes et al. (1999) reported that 17β-estradiol and estrone were frequently detected in 16 municipal German sewage treatment plants (STP) and 10 Canadian STPs, and the average concentrations of 17β-estradiol and estrone were 0.015 μg l^{-1} and 0.027 μg l^{-1} in those STPs, respectively.

After steroidal hormones are released into the environment, they undergo several fate and transport processes, which have been reported by various researchers (Braga et al., 2005; Campbell et al., 2006; Tamagama et al., 2006). For 17β-estradiol, the water solubility is reported

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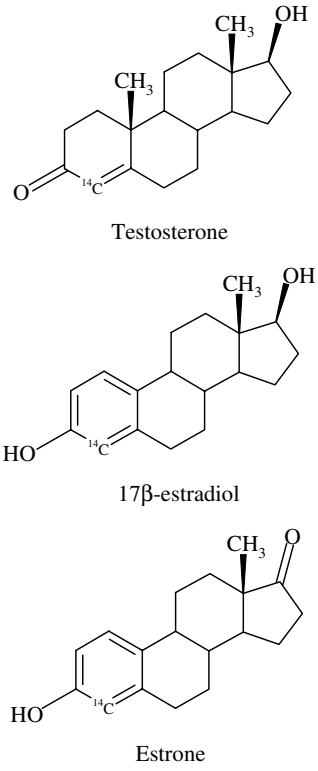

Fig. 1. Structures of testosterone, 17β-estradiol, and estrone. The radiolabeled carbons were located in the carbon position 4.

to be 13 mg l^{-1} at 20 °C, and the logarithm of its octanol–water partition coefficient is 3.94 (Lai et al., 2000). 17β-Estradiol and testosterone do bind strongly to the organic phase of soil particles and are not dissolved or transported in soil water (Casey et al., 2003, 2004; Anderson et al., 2005). Under laboratory conditions, Schicksnus and Mueller-Goymann (2000) reported that the solubility of 17β-estradiol is a function of ionic strength and pH of the solution.

Jacobsen et al. (2005) conducted various laboratory studies to investigate the fate of 17β-estradiol and testosterone in various organic matrices (e.g., manure and biosolids) under aerobic conditions. Their results indicated that at the end of 6 d of incubation, 47% and 36% of testosterone in silt loam soil was mineralized to CO_2 in manured and unmanured treatments, respectively; however, no mineralization occurred in sterilized soil. These results also demonstrated that 17β-estradiol and testosterone were degraded more slowly at low temperature than at high temperature. Different sterilization experiments indicated that there was no mineralization of testosterone and 17β-estradiol in sterile soils, which suggested that microbial processes play a key role in hormone degradation under aerobic conditions. Hakk et al. (2005) performed incubation experiments under aerobic conditions to assess the water extractability of 17β-estradiol and testosterone in chicken manure compost with 60% moisture. They concluded that the extractability of 17β-estradiol and testosterone decreased with time, and the first-order degradation rate constant for 17β-estradiol was 0.010 d^{-1}, which was slightly lower than that for testosterone, 0.015 d^{-1}. Colucci et al. (2001) conducted various laboratory incubations experiments under aerobic conditions to investigate the persistence and pathways of 17β-estradiol in agricultural soils. After 3 d of incubation at 30 °C, they found that 17.1% of 1 mg kg^{-1} 17β-estradiol is mineralized to CO_2 in sandy loam soil with a moisture content of 0.13 g g^{-1} and about 20% of 10 mg kg^{-1} 17β-estradiol is mineralized to CO_2 in the same soil with a moisture content of 0.15 g g^{-1}. In comparison, only 0.4% of 10 mg kg^{-1} 17β-estradiol is mineralized in air-dried sandy loam soil under the same conditions. Their results also indicate that in a loam soil with a moisture content of 0.13 g g^{-1}, 14.7% and 3.6% of 17β-estradiol is mineralized to CO_2 at 37 °C and 4 °C, respectively, which suggested that the rate of dissipation of 17β-estradiol is significantly different at different soil temperatures.

Although some studies have identified the persistence of 17β-estradiol and testosterone in various soils, the fate of these two hormones in the environment is still not fully understood, especially in sub-surface environments where anaerobic conditions often occur. Up to now, no such experiments have been done to study the fate of steroidal hormones under anaerobic conditions. The goal of this research was to investigate the fate and occurrence of 17β-estradiol and testosterone in agricultural soils under aerobic and anaerobic conditions using incubation experiments. These experiments can help identify the persistence, occurrence, and fate of hormones. Also, the potential impacts on soils and subsurface water resources can be assessed using these experiments.

2. Materials and methods

2.1. Sample Collection

Samples of Hamar soil series (sandy, mixed, frigid typic endoaquolls) were collected from the surface (A) horizon. All ground covers were removed before sampling. The following soil physical properties were determined: bulk density of 1.54 g cm^{-3}; porosity of 0.42; organic matter of 2.23%; 14.0% clay; 19.0% silt; and 67% sand. Soil samples were transported to the laboratory, where they were then immediately stored at 4 °C for periods of up to six months prior to experimentation.

2.2. Chemicals

[4-^{14}C]-radiolabeled 17β-estradiol and testosterone (>99% by TLC) were purchased from American Radiolabeled Chemicals, St. Louis, MO, and stored at 4 °C before use. Bray's solution (Bray, 1960) was used to trap $^{14}CH_4$ released from soils under anaerobic conditions. Bray's solution was made of 60 g of naphthalene (Sigma-Aldrich, St. Louis, MO), 4 g of 2,5-diphenyloxazole (Packard

Chemicals, Meriden, CT), 0.2 g of 1,4-bis(5-phenyloxazo-2-ly)benzene (Sigma-Aldrich, St. Louis, MO), 100 ml of methanol (>99%; Fisher Chemical, Fairlawn, NJ), 20 ml of ethylene glycol (Fisher Chemical, Fairlawn, NJ), and 880 ml of *p*-dioxane (Burdick & Jackson, Midland, MI). The Bray's solution was stored at room temperature (25 °C) over periods of up to one month. Bulk packing material [Porapak type Q, 100–200 mesh (Waters Corporation, Milford, MA)] was used to trap other volatile organic compounds. The Porapak was cleaned by washing in the following order: 45 ml of MeOH, 45 ml of acetone (>99%; Fisher Chemical, Fairlawn, NJ), 45 ml of MeOH, and 45 ml of deionized H_2O, and then was air-dried at room temperature.

2.3. Incubation experiments

The persistence and fate of 17β-estradiol and testosterone were each investigated under the following four soil microcosms: native soil under aerobic conditions, native soil under anaerobic conditions, autoclaved soil under aerobic conditions, and autoclaved soil under anaerobic conditions. Autoclaved soils were prepared by autoclaving the soils for 40 min at 122 °C.

To create solution concentration of 0.052 mg l^{-1} for testosterone and 1.5 mg l^{-1} for 17β-estradiol, 75 μg of [4-^{14}C]-radiolabeled 17β-estradiol (approximately 1 000 000 dpm) or 2.62 μg of [4-^{14}C]-radiolabeled testosterone (approximately 1 000 000 dpm) per 200 g of soil in approximately 50 ml of 0.01 M $CaCl_2$ (Sigma-Aldrich, St. Louis, MO) was gradually added to the native and autoclaved soils, which adjusted the soil moisture content to 100% water-holding capacity (saturated soil but not slurries) at the beginning of the incubation. These concentrations were chosen because they have been found in animal manures applied to agricultural fields (Shore and Shemesh, 2003; Lorenzen et al., 2004). The weak salt solution (i.e., 0.01 M $CaCl_2$) was used so soil aggregates would not be dispersed. The volumetric fraction of methanol in each initial stock solution was less than 1%, which has been previously shown to not significantly affect the sorption of organic contaminants to soil (Wauchope and Koskinen, 1983). In addition, 500 mg $HgCl_2$ (Fisher Chemical, Fairlawn, NJ) per kg soil was added to the autoclaved soils to inhibit any potential airborne bacterial activities (Trevors, 1996).

About 210 g of soil, not sieved or air-dried, was added to a 250 ml glass flask (Fig. 2). The flask was then covered with aluminum and sealed with a rubber stopper to eliminate the possibility of photodegradation. Tygon® tubing (6 mm inner diameter) was used for all connections. Moist air was used as the carrier gas under aerobic conditions, and humidified helium gas was used as the carrier gas under anaerobic conditions. The carrier gas was humidified

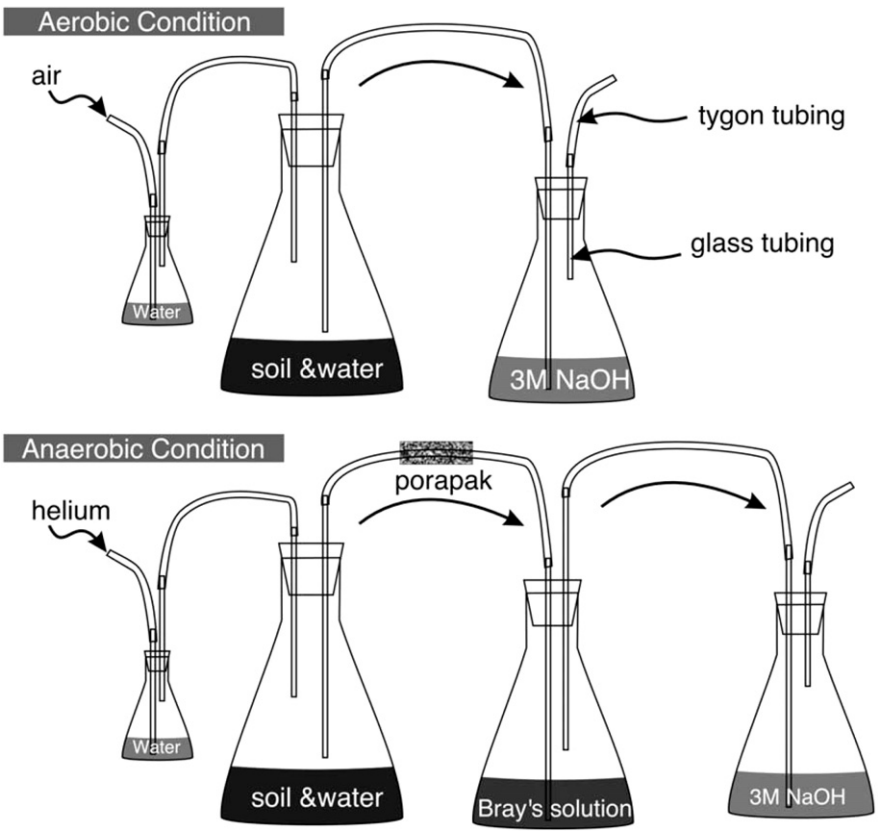

Fig. 2. Schematic of incubation experiments under aerobic and anaerobic conditions.

to maintain a constant soil moisture in either aerobic or anaerobic conditions. In the aerobic systems (Fig. 2), the outlet gas from the soil-filled flask was passed through a tube that was connected to a 200 ml glass flask containing 120 ml of 3 M NaOH, which trapped $^{14}CO_2$. In the anaerobic systems (Fig. 2), the outlet gas from the soil-filled flask was first passed through a hand-packed Porapak column (i.d. 0.5 cm, length 6 cm), which trapped the ^{14}C-labeled volatile organic compounds, except $^{14}CH_4$. After passing through the Porapak column, the outlet gas continued through two traps. The first trap contained 120 ml of Bray's solution, which trapped $^{14}CH_4$, and the second trap contained 120 ml of 3 M NaOH, which trapped $^{14}CO_2$. On hours 1, 2, 3, 4, 5, 30, 54, and 132, two 500 μl aliquots were removed from each of the NaOH and Bray's solutions and were dissolved in 16 ml of scintillation cocktail, allowed to settle for at least 48 h at room temperature, and assayed for radioactivity by liquid scintillation counting (LSC; 1900 CA scintillation counter, Packard, Downers Grove, IL). At 132 h, the experiment was stopped and the Porapak was washed in the following order: 3 ml of H_2O, 3 ml of MeOH, and 3 ml of acetone. Aliquots of 100 μl were removed from each of these sequential washings and assayed for radioactivity by LSC.

Additionally, extractability of resident ^{14}C in the soil was determined. This was accomplished by extracting the soil first with water (three times the soil moisture content), and then with acetone (three times the soil moisture content). Aliquots of 500 μl were removed from the two extracts and assayed for radioactivity by LSC. Analysis for metabolites was also done on the two extracts using thin-layer chromatography (TLC) (System 2000 Imaging Scanner (Bioscan, Inc., Washington DC)). The TLC was done using silica gel plates (250 μm; Whatman Lab. Div., Clinton, NJ) developed with methylene chloride:ether: hexane (2:3:4).

Following the solvent extraction, the remaining non-extractable ^{14}C was fractionated as described by Kaplan and Kaplan (1982) to determine the distribution of non-extractable ^{14}C among the various organic matter fractions (e.g., humic acid, fulvic acid, and humin). This was done in the following order: (1) air-drying and grinding the soil, (2) placing 50 g of dry soil into a 500 ml cylinder, (3) washing the soil with 200 ml of 0.1 N HCl, (4) extracting the soil with 200 ml of 0.5 N NaOH and 0.1 N $Na_4P_2O_7 \cdot 10H_2O$, respectively, (5) combining the two fractions of NaOH and $Na_4P_2O_7 \cdot 10H_2O$, and (6) adjusting the combined solution to pH 3.0. The insoluble materials that remained after steps (4) and (6) were considered to be associated with humin and humic acid, respectively. The radioactivity associated with these two organic matter fractions (i.e., humin and humic acid) were assayed by combustion analysis on a Packard Model 307 Oxidizer (Packard Chemicals, Meridan, CT). The soluble material after step (6) was considered to be associated with fulvic acid, and was assayed for radioactivity by LSC.

2.4. Determining mineralization rate

A first-order mineralization rate constant, k (h^{-1}), was determined for the removal of steroidal hormones (e.g., testosterone and 17β-estradiol) by mineralization to CO_2. The rate expression can be written in the following way:

$$\frac{\partial(^{14}CO_2)}{\partial t} = k^{14}C(t) \quad (1)$$

where $^{14}C(t)$ is the concentration of steroid hormones (ng g^{-1}) at time t (h), and $^{14}CO_2$ is the concentration of carbon dioxide (ng g^{-1}) at time t. Integrated, Eq. (1) can be written as:

$$1 = e^{-kt} + \frac{(^{14}CO_2)}{^{14}C(0)} \quad (2)$$

where $^{14}C(0)$ is the initial concentration of steroid hormones and $(^{14}CO_2)/^{14}C(0)$ is the fraction of initial ^{14}C as CO_2 in NaOH solution.

3. Results and discussion

Under aerobic and anaerobic conditions, 6% and 0.9% of 17β-estradiol was mineralized to $^{14}CO_2$ in native soil, respectively (Table 1). This is compared to 63% and 46% of testosterone (Table 2) that was mineralized to $^{14}CO_2$ under aerobic and anaerobic conditions, respectively. In native soil, under anaerobic conditions, there were no $^{14}CH_4$ or other ^{14}C-labeled volatile organic compounds detected for the 17β-estradiol incubation experiments. However, 2% of testosterone was methanogenized to $^{14}CH_4$ in native soil under the same anaerobic conditions. Experiments were also done with autoclaved soil to elucidate whether the conversion of 17β-estradiol or testosterone to volatile compounds was biologically facilitated. These experiments in autoclaved soils showed that no 17β-estradiol or testosterone was converted to $^{14}CO_2$, $^{14}CH_4$, or other ^{14}C-labeled volatile compounds (Tables 1 and 2), which indicated that microbes played an important role in the transformation/degradation of both these hormones. These findings were similar to those reported by Colucci et al. (2001), who conducted a series of incubation experiments to investigate the persistence of hormones in soils under aerobic condition, and found that 8.2% of initial 17β-estradiol was mineralized to CO_2 at 30 °C. Jacobsen et al. (2005) also reported that 47% and 36% of testosterone (in silt loam soil with 0.076 g g^{-1} moisture at 30 °C) was mineralized to CO_2 in manured and unmanured treatments, respectively, following 6 d incubations under aerobic conditions. Layton et al. (2000) found that 70–80% of 17β-estradiol and 55–65% of testosterone was mineralized to CO_2 in biosolids under aerobic conditions.

3.1. Mineralization rates

The first-order mineralization rate constants, k [Eqs. (1) and (2)], for testosterone in native soil under aerobic and

Table 1
Mineralization and fermentation fraction of original ^{14}C labeled 17β-estradiol in native and autoclaved soils under aerobic and anaerobic conditions after 5-d duration

	Aerobic condition		Anaerobic condition	
	Native soil (%)	Autoclaved soil (%)	Native soil (%)	Autoclaved soil (%)
Trapped ^{14}C				
$^{14}CO_2$	6	0.2	0.9	0
$^{14}CH_4$	N/A	N/A	0	0
Extractable ^{14}C				
H_2O	2	2	2	1
Acetone	10	26	17	23
Subtotal	12	28	19	24
Non-extractable ^{14}C				
Humic acids	37	31	37	24
Fluvic acids	17	13	22	15
Humin	19	23	11	11
Subtotal	73	67	70	50
Other ^{14}C volatile organic compounds	N/A	N/A	0	0
Total ^{14}C recovered	91	95.2	89.9	74

Table 2
Mineralization and fermentation fraction of original ^{14}C labeled testosterone in native and autoclaved soils under aerobic and anaerobic conditions after 5-d duration

	Aerobic condition		Anaerobic condition	
	Native soil (%)	Autoclaved soil (%)	Native soil (%)	Autoclaved soil (%)
Trapped ^{14}C				
$^{14}CO_2$	63	0.2	46	0
$^{14}CH_4$	N/A	N/A	2	0
Extractable ^{14}C				
H_2O	0.4	0.2	0	0.5
Acetone	3	25	16	37
Subtotal	3.4	25.2	16	37.5
Non-extractable ^{14}C				
Humic acids	3	11	5	9
Fluvic acids	9	2	0	3
Humin	7	41	20	37
Subtotal	19	54	25	49
Other ^{14}C volatile organic compounds	N/A	N/A	0	0
Total ^{14}C recovered	85.4	79.4	89	86.5

anaerobic conditions were 0.012 h^{-1} and 0.004 h^{-1}, respectively. For 17β-estradiol in native soil under aerobic and anaerobic conditions, k was 0.0006 h^{-1} and 0.0001 h^{-1}, respectively. Under aerobic conditions at 22–25 °C, Layton et al. (2000) found that k was 0.912 h^{-1} for testosterone and 0.252 h^{-1} for 17β-estradiol in municipal biosolids (obtained from the wastewater treatment systems). The differences of k values between the Layton et al. (2000) study and the present study may have been caused by the potentially higher microbial population densities of the biosolids used in their studies.

Even though the initial concentration of testosterone (0.052 mg l^{-1}) was lower than 17β-estradiol (1.5 mg l^{-1}), the testosterone mineralization rates were still much higher than 17β-estradiol under both aerobic and anaerobic conditions. One reason that may contribute to the higher mineralization rate of testosterone is a greater bioavailability of testosterone to microbes compared to 17β-estradiol. Harms and Bosma (1997) reviewed many studies on pollutant degradation and concluded that organic contaminants, including non-aqueous phase liquids, solid compounds, and sorbed substrates have to be dissolved or desorbed into the aqueous phase, to be available to microbes. Because 17β-estradiol is more strongly bound to the soil particles than testosterone (Shemesh and Shore, 1994; Casey et al., 2003, 2004), it would have a lower bioavailability potential than testosterone, which may explain its lower mineralization rate. Another factor that may contribute to the difference in the mineralization rates is that the aromatic structure of 17β-estradiol is more stable

(higher activation energy of ring cleavage) than the cyclohexane ring of testosterone (Fig. 1).

Figs. 3 and 4 also indicated that the total mineralization of 17β-estradiol and testosterone were generally higher under aerobic conditions than under anaerobic conditions. The difference in total mineralization of 17β-estradiol and testosterone under aerobic compared anaerobic conditions may be due to several reasons. (1) Different degradation pathways were involved under aerobic compared to anaerobic conditions. Horrison et al. (2001) reported that in the same soil, *p*-cresol, a phenolic compound, was biotransformed via a catecholic pathway under aerobic conditions; however, under anaerobic conditions it was biotransformed via reductive pathway. (2) Electron (e^-) acceptors are readily present under aerobic compared to anaerobic conditions. Korom (1992) reviewed many studies on biological processes in groundwater, and concluded that when oxygen is present, then microbes can create energy by oxidizing carbohydrate and reducing oxygen. When oxygen supplies become limited, then microbes will seek alternative

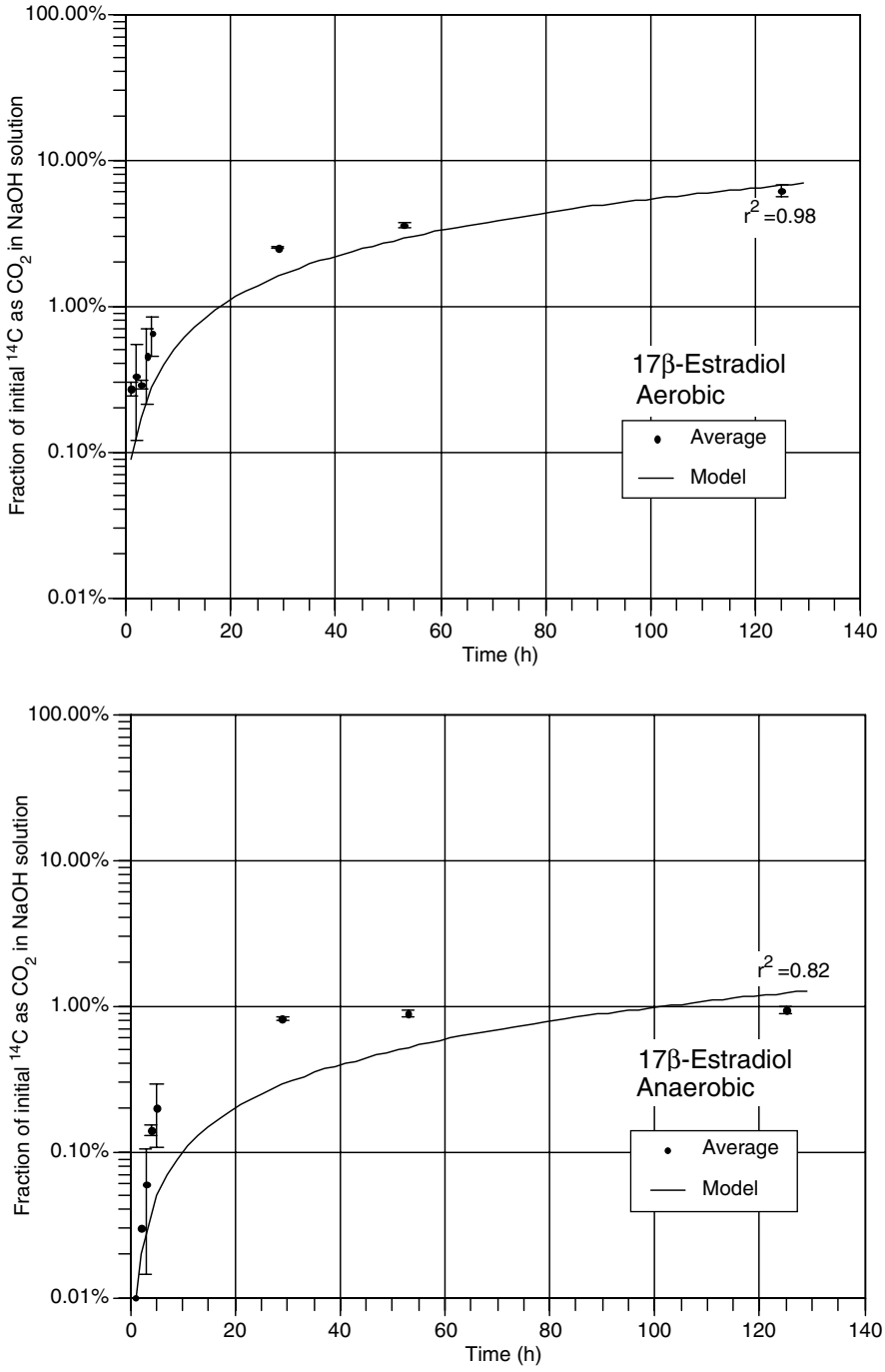

Fig. 3. The production of $^{14}CO_2$ from the mineralization of ^{14}C-17β-estradiol in native soil under aerobic and anaerobic conditions through a 5-d period of time. Symbols and error bars represent the average and range of the measured values, respectively.

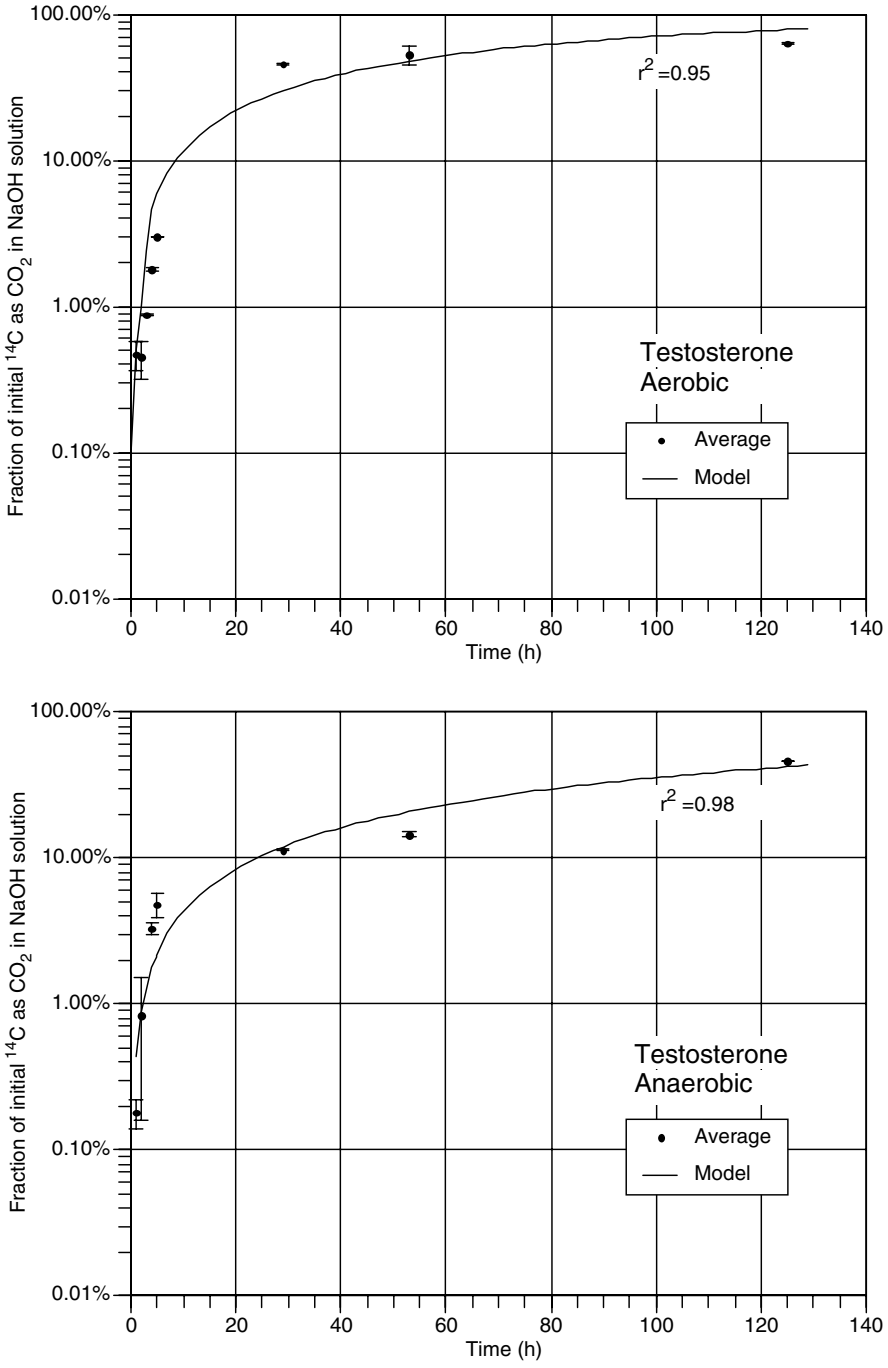

Fig. 4. The production of $^{14}CO_2$ from the mineralization of ^{14}C-testosterone in native soil under aerobic and anaerobic conditions through a 5-d period of time. Symbols and error bars represent the average and range of the measured values, respectively.

terminal e^- acceptors in the order: NO_3^-, Mn(IV), Fe(III), and SO_4^{2-}. The growth of microbes might be limited due to the lower level of e^- acceptor under anaerobic conditions thus reducing the metabolism of these hormones. (3) Different microorganism species were involved in mineralization under aerobic compared to anaerobic conditions. The different microbes may have different growth rates and may utilize different degradation pathways, thus resulting in different metabolism rates.

3.2. Microbial degradation

For testosterone, the TLC analysis on the native soil extracts (i.e., water and acetone) indicated that 83% and 87% of the extractable ^{14}C (data not shown) was metabolite under aerobic and anaerobic conditions, respectively. However, when the soil was autoclaved, all of the extractable ^{14}C material was parent testosterone (Table 3) under both aerobic and anaerobic conditions. These results

Table 3
Major metabolites of 17β-estradiol and testosterone under aerobic and anaerobic conditions

	Aerobic condition		Anaerobic condition	
	Native soil	Autoclaved soil	Native soil	Autoclaved soil
17β-Estradiol	An unidentified polar compound (63%[a])	An unidentified polar compound (12%)	Estrone (28%) and an unidentified polar compound (61%)	An unidentified polar compound (9%)
Testosterone	An unidentified polar compound (87%)	NA[b]	An unidentified polar compound (83%)	NA

[a] Percentage of the extractable ^{14}C.
[b] N/A indicates that there is no metabolite detected during the experiments.

strongly suggest that testosterone mineralization was biological since the parent testosterone persisted in the autoclaved soil but was transformed in the native soil where microbes were present. Also, for native soil under aerobic and anaerobic conditions, there was an unidentified metabolite detected that was less soluble in the TLC mobile phase than testosterone.

For 17β-estradiol, the TLC analysis on the native soil extracts (i.e., water and acetone) indicated that 100% and 89% of the extractable ^{14}C material (data not shown) was determined to be a metabolite under aerobic and anaerobic conditions, respectively. However, when the soil was autoclaved, 88% and 91% of the extractable ^{14}C material remained as parent 17β-estradiol under aerobic and anaerobic conditions, respectively (Table 3). An unidentified polar compound was found to be the major metabolite in the autoclaved soil under anaerobic conditions (Table 3). This unidentified metabolite was also found in native soil under anaerobic conditions, and in both native and autoclaved soils under aerobic conditions (Table 3). In the native soil under anaerobic conditions, degradation of 17β-estradiol was accompanied by another radiolabeled product, which had a TLC retention time identical to the estrone standard. This apparent estrone metabolite was not found in the autoclaved soils under aerobic and anaerobic conditions, and in the native soil under aerobic condition. Several other studies have found that 17β-estradiol is adsorbed rapidly and dissipated within a few hours in agricultural soils under aerobic conditions (Colucci et al., 2001; Casey et al., 2003; Jacobsen et al., 2005). Under aerobic conditions in loam soil, Colucci et al. (2001) found that 17β-estradiol could be oxidized to estrone within 6 h, and thereafter estone was not detected. This result was consistent with the results of the current study where no estrone was detected in the native soil under aerobic conditions. Taken together, these results suggest the following: (1) 17β-estradiol could be transformed into (an) unidentified polar compound(s) in non-biological processes; (2) 17β-estradiol is not degraded to estrone by any physical–chemical processes but exclusively by biological processes; and (3) estrone could be further mineralized to $^{14}CO_2$ or degraded to some polar compounds under aerobic conditions but not under anaerobic condition. The results from the current study are also different from other results reported by Colucci et al. (2001) and Jacobsen et al. (2005), who found that 17β-estradiol could be oxidized to estrone without microorganisms. One reason for this discrepancy may have been the inadvertent introduction of microorganisms, possibly airborne, during their incubation experiments, while $HgCl_2$ was added in the autoclaved soils of the current study to prevent possible microorganism growth.

All these results indicated that 17β-estradiol can be transformed to some polar compound in processes that do not involve microbial activities, and testosterone can only be degraded by microbial processes. These results elucidate that degradation occurs in the liquid phase, something previous studies did not know (Casey et al., 2003, 2004; Das et al., 2004; Yu et al., 2004). Colucci et al. (2001) found that mineralization of 17β-estradiol depended highly on soil moisture content. They found that 20% of 17β-estradiol was mineralized in sandy loam soil with a moisture content of 15%, while only 0.4% of 17β-estradiol was mineralized in the air-dried soil. As discussed earlier, contaminants have to be dissolved or desorbed into the aqueous phase so that the microorganism can consume them. An improved understanding of the desorption of steroidal hormones on the solid–aqueous interface, diffusion through the aqueous phase to the microorganisms, and the degradation of steroidal hormones by microbial processes, may provide better prediction of the fate of steroidal hormones in soil–water systems and help to develop strategies for remediation of steroidal hormones.

3.3. Extractability

Following 5 d of incubation, 70–73% and 50–67% of 17β-estradiol (Table 1) was non-extractable from the native and autoclaved soils, respectively. Also, after 5 d incubation 19–25% and 49–54% of testosterone (Table 2) was non-extractable from the native and autoclaved soils, respectively. Also, approximately 12–19% of 17β-estradiol (Table 1) and 3–16% of testosterone (Table 2) were extracted water and acetone in native soil; however, 24–28% of 17β-estradiol (Table 1) and 25–38% of testosterone (Table 2) were extractable in autoclaved soils. These results (i.e., the autoclaved soil had a higher extractable fraction) indicate that microorganisms reduce the concentration of extractable ^{14}C by the hormone degradation process. Another possible explanation for the different proportion of extractable hormone may have resulted from autoclaving, which was used to sterilize the soil. Trevors (1996)

reported that autoclaved soil might destroy soil structure and change soil physical (e.g., pore size) and chemical properties, which would change the sorptive sites, and sorption properties of testosterone.

3.4. Distribution of non-extractable hormones among the humic substances

The non-extractable hormones remaining in the soil after solvent extraction were mostly associated with humic substances, the naturally occurring organic matter, which represents more than 50% of the OM in soils (Kohl and Rice, 1996). Tables 1 and 2 showed that approximately 50–73% of 17β-estradiol and its metabolites, and 19–55% of testosterone and its metabolites were bound by humic substances. These results also indicated that most of non-extractable ^{14}C for 17β-estradiol was associated with the humic acids, compared to testosterone, which was mostly associated with the humin fraction. This difference may be due to the reactive phenolic group, which is on 17β-estradiol and estrone but not on testosterone. Yu et al. (2004) suggests that this phenolic group of 17β-estradiol and estrone can interact with humic acids or mineral surfaces via hydrogen and covalent bonds. This may explain the distribution differences among the humic substances for the androgens and estrogens. Another possible explanation would be the hydrolysis of any bonds between the estrogen phenolic group and the soil matrix (Yu et al., 2004) during the soil organic fractionation process. The Kaplan and Kaplan extraction, described in the methods section, changes the acidity of the solution, which may hydrolyze the bonds of the estrogens or their metabolites to the soil matrix. This bond hydrolysis may also contribute to the apparent difference between the testosterone and estrogen distributions among the organic fractions.

Humic substances have brown to black color and relatively high molecular weight ranging from several hundreds to tens of thousands (Krishnamurthy, 1992), and can be divided into three groups of compounds based on their solubility in bases and acids (Islam et al., 2005). Fulvic acids are smaller molecular weight organic molecules (around 2000 Dalton) and are soluble in both acids and bases. Humic acids are large molecular weigh organic molecules (5000–100 000 Dalton) and are only soluble in bases. Humin have the greatest molecular weight of the humic substances (around 300 000 Dalton) and are insoluble in both acids and bases. McGechan and Lewis (2002) reported that humic substances can mobilize and act as a mobile or flowing solid phase. These mobile humic substances can sorb contaminants in a fashion similar to the immobile or stagnant solid phase, but can migrate at rates similar to, or even greater than, the mobile aqueous phase. Johnson and Amy (1995) reported that humic substances can significantly enhance desorption of polycyclic aromatic hydrocarbons (PAH) in low organic carbon aquifer sediments. Sabljic et al. (1989) and Schlautman and Morgan (1993) suggested that the binding of PCBs and PAH (e.g., anthracene, pyrene, and perylene) by humic materials depended on the size of the solute molecule as well as its hydrophobicity. Haitzer et al. (1999) did various experiments to investigate the effects of humic substances on the bioconcentration and toxicity of PAHs, which depended on the chemical properties of humic substances (e.g., polarity, aromaticity, and bulk properties). Therefore, an improved understanding of the binding and interactions between steroid hormones by humic substances may help to predict the bioavailability of hormones in the agricultural soils.

3.5. Environmental significance

On the basis of present study, it may be surmised that biological processes, rather than physical–chemical processes, play a dominant role in the degradation or transformation of 17β-estradiol and testosterone. The decline in bioavailability and extractability of 17β-estradiol and testosterone through time suggests the risk of hormones in the environment may be overestimated. Chung and Alexander (1998) did several experiments to study the sequestration and bioavailability of organic compounds in soils, and found that there was a positive correlation between the decrease in bioavailability and the decrease in extractability of phenanthrene and atrazine 120 d after the two chemicals (1.0 and 6.0 $\mu g\ g^{-1}$ dry soil for phenanthrene and atrazine) were applied to soil. They also reported that this correlation increased if the decline in extractability of organic compounds in soils was greater than 40%.

The apparent result that most of non-extractable hormones were associated with humic substances indicated that hormones are more degradable in soils with low organic content due to the high extractability and high bioavailability. However, the high extractability may also increase the risk of hormone transport to groundwater or surface water through runoff and subsurface draining systems. Other studies (Casey et al., 2003, 2004) have also indicated that if hormones were applied to the soil surface, then nearly all would be retained in the topsoil. Taken together, the risk of hormones in the environment is small and typical manure best management practices for soil nitrogen or phosphorous requirements would go far in further reducing their potential impact on the environment.

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